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COMMUNICATION

The Induction of Cytolytic T Lymphocytes with Syngeneic Trinitrophenyl-Coupled Membranes[1]

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Recently we have demonstrated the induction of allogeneic murine cytolytic T lymphocytes (CTL)[3] using purified plasma membranes rather than intact cells as stimulatory agents (1). In this report we extend the use of such subcellular preparations to study the requirements for hapten-specific syngeneic CTL induction. Membranes prepared from trinitrophenyl (TNP) coupled syngeneic tumor cells retain the ability to stimulate both a primary and secondary CTL response. The CTL that are generated are restricted in their lysis to target cells bearing the same H-2 antigens as those present on the TNP-coupled stimulating membranes.

MATERIALS AND METHODS

All materials and methods used in the *in vitro* induction and assay of TNP specific CTL are as previously described (2). Briefly, 7×10^6 spleen cells from nonimmune or immune mice were co-cultured with x-irradiated, TNP-coupled spleen cells or TNP-coupled membranes. After 5 days of culture cells were harvested and cytolytic activity was assessed in a 4-hr assay against 10^4 ^{51}Cr-labeled TNP-coupled tumor targets or LPS blast cell targets. Immune spleen cells were obtained by priming mice subcutaneously with 2×10^7 TNP-coupled autologous spleen cells 2 weeks before *in vitro* culture. Membranes used in stimulation of CTL were prepared from TNP coupled DBA/2 mastocytoma P815 (H-2^d) tumor cells or from TNP-coupled C57BL/6 (B6) leukemia EL-4 (H-2^b) tumor cells. Purified plasma membranes were used for CTL induction in the experiment described in Table I. The results presented in Tables II and III were obtained by using partially purified plasma membranes referred to as "high speed pellet" in Reference 1. Spontaneous ^{51}Cr release ranged from 30 to 39% for LPS-induced blast cell targets and from 11 to 19% for tumor cell targets.

RESULTS

Plasma membranes prepared from TNP-modified tumor cells were tested for their ability to stimulate both primary and secondary CTL responses by using H-2 syngeneic responder cells. As demonstrated in Tables I and II, such membranes were active in the stimulation of primary and secondary hapten specific CTL. CTL generated by coupled membranes are similar in specificity to those generated by coupled cells in that they preferentially lyse syngeneic target cells (2–4). These membranes stimulated variable amounts of cross-reactive lysis on TNP coupled allogeneic target cells. B6 spleen cells stimulated with TNP-EL-4 membranes did not lyse targets that did not bear TNP. Lytic activity was not induced when B6 spleen cells were co-cultured with uncoupled EL-4 membranes.

It has been recently demonstrated (5, 6) that cells incubated with TNP-coupled proteins are capable of stimulating a hapten-specific cytolytic response that is restricted to target cells that are H-2 identical with the responder cell population. Therefore, it was important to determine if stimulation by the membranes was dependent on the H-2 antigen present on the membrane, or whether the membrane proteins were simply contributing the hapten that was then recognized in conjunction with the H-2 antigens of the responder cell population. B6D2F$_1$ (H-$2^{b/d}$) immune spleen cells were stimulated with either TNP-EL-4 membranes or TNP-P815 membranes and the specificity of the resultant CTL was studied. It would be expected that if the H-2 present on the membranes did not influence the specificity of the CTL, then in either case the CTL would lyse both B6-TNP (H-2^b) and B10.D2-TNP (H-2^d) targets to a similar extent. As is shown in Table III the CTL preferentially lyse target cells that bear the same H-2 antigens as the TNP-membranes used in CTL stimulation. Similar specificity was obtained with CTL resulting from stimulation of nonimmune B6D2F$_1$ spleen cells (Table II). These results indicate that both the TNP and the H-2 antigens present on the membranes determine the specificity of the CTL population.

DISCUSSION

The results described above extend the use of subcellular material to the study of CTL recognition in a chemically modified syngeneic system. The results demonstrate the capacity of membranes prepared from TNP-modified tumor cells to induce primary and secondary CTL having the same specificity as CTL that are induced by TNP-coupled cells. The ability to stimulate B6D2F$_1$ CTL that are restricted in their recognition to the H-2 antigens present on the stimulating membrane

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[3] Abbreviations used in this paper: CTL, cytolytic T lymphocytes; H-2, histocompatibility complex-2; B6D2F$_1$, (C57BL/6 × DBA/2)F$_1$; B6, C57BL/6.